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Blights of Apple and Pear Trees

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BLIGHTS OF APPLE AND PEAR TREES

BY

HOWARD SPURR HAMMOND

B. A. Ohio State University, 1908

THESIS

Submitted in Partial Fulfillment of the Requirements for the

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IN

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

ENTITLED *Howard Spurr Hammond*
Blights of Apple and Pear Trees

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

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J. Burrill
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In Charge of Major Work
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Recommendation concurred in:

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BLIGHTS OF APPLE AND PEAR TREES

I. A Comparison of the Morphologic and Physiologic Characters of Bacillus amylovorus (Burr.) Trevisan with those of similar organisms infesting the Grimes' Golden and Willow Twig varieties of Apple.

INTRODUCTION

The object of this paper, as the title suggests, is to give an account of the morphologic and physiologic characters of Bacillus amylovorus (Burr.) Trevisan with those of similar organisms infesting two varieties of apple. A brief history of some of the various theories as to the cause of the blight in the pear is given, also a brief description of the general appearance and distinguishing characters of the effects of the bacteria in the apple.

Special attention is called to the growth of these various organisms in different non-synthetic and synthetic media, the results of which form the basis of the paper and which show a difference in some instances between the growth of Bacillus amylovorus (Burr.) Trevisan, the pear blight bacillus, and that of the organisms obtained from the two varieties of apple under consideration. These variations in cultural characteristics raise the question whether the organisms infesting the apple and the pear are the same, as some writers

have stated, or whether they are really two distinct species. This question can only be settled after a series of inoculation experiments have been carried on.

HISTORICAL

Our knowledge of the disease of the pear and apple trees commonly known as "Fire blight" in the pear and "Twig blight" in the apple, is by no means of recent origin, for Sackett¹ (32) tells us that "it dates back to the time of William Denning, who first reported the trouble from the Highlands of the Hudson in 1770". In an article written by Denning² (20) published in 1794 he described the cause as due to an insect boring in the trunk of the tree and causing the parts beyond to die.

The earliest book containing a critical account of the disease is that of Coxe, published in 1817, nearly a century ago. He characterized the disease in the following words: "That species of blight which is sometimes called fire blight, frequently destroys trees in the fullest apparent vigor and health, in a few hours, turning the leaves suddenly brown, as if they had passed through a hot flame, and causing a morbid matter to exude from the pores of the bark, of a black ferru-

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1. Mich. Agr. Expt. Sta. Bull. No. 230. 205-210. 1905.

2. Trans. Soc. Prom. Agr. etc. Vol. 2. 219-222. 1794.

ginous appearance; this happens through the whole course of the warm season more frequently in weather both hot and moist".

Arthur¹ (2) states that " the years 1826 and 1832 were notable in horticultural circles for the increased prevalence of the disease; but it was in 1844 that the most widespread and fatal epidemic that the country has ever yet (1886) known, occurred." Probably no one subject received more attention at horticultural gatherings, or more space in periodicals devoted to horticulture than did pear blight, during the next forty or fifty years, which Downing has justly termed " the monstrous malady of the pear".

Numerous theories were advanced as to the cause and many remedies offered only to be overthrown by subsequent observations.

The first theory to gain many adherents was the insect theory, which was advanced about the time of Coxe's article. It had its foundations in the discovery of a small brown beetle, known to science as Xyleborus pyri, which bored into the twigs and branches of the tree and caused the parts beyond to die.

The next theory, and probably the most widely accepted of the earlier ones, was the frozen sap theory advanced in 1844 by the Rev. H. W. Beecher of Indiana and independently in 1845 by Downing (21) in his work on Fruits and Fruit Trees

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1. Proc. Phila. Acad. Nat. Sci. 322-341. Pl. III. 1886.

of North America. The latter is usually known as the author of it. This theory held that the disease was due to the freezing and thawing of the sap, which thus lost its vitality and became dark, discolored, and poisonous to the plant. Because this theory explained many phenomena connected with the disease in a very satisfactory manner it gained great popularity especially in the western states.

The fungous theory was first advanced in 1863 by Salisbury¹ (33) of Ohio, who published an illustrated account of his observations. He concluded the disease was of fungous origin and named the fungus Sphaerotheca pyri.

In 1868 Meehan, editor of the Gardener's Monthly, published accounts of Hunt's² (25) work upon microscopic examination of blighted pear twigs, which was undertaken at Meehan's request. Dr. Hunt confirmed the opinion of Salisbury that it was due to a fungus, but he did not decide as to its specific characteristics. The following, ~~is~~ taken from Hunt's report of his investigations as recorded in Burrill's³ address on "The Anthrax of Fruit Trees", shows how nearly he approached the cause of the disease. "It attacks the bark and outside of the leaves and young fruit first causing changes in the cells, in these locations resembling much those pigmentary cell changes which differentiate the negro from the so called white man.

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1. Ohio Agr. Rept. 1863., p. 450.
2. Gardener's Monthly 17: 245. 1875.
3. Proc. Amer. Assoc. 19: 583-597. 1880.

The cell contents, normally transparent, are changed into extremely minute pigment granules which fill the cells and give the characteristic color and smell which marks the disease. Moreover, minute drops of viscid offensive liquid come out on the surface. From the cambium layer the fungus travels towards the interior of the stem through the medullary rays chiefly, and here I find those round bodies which in our ignorance we often call spores. The ducts which ascend the stem are often obstructed with similar bodies and aggregated pigment granules. This is all I know about the subject. I cannot venture to name the fungus."

Because of the fact that trees infected with blight often attract attention after a thunder storm, the belief that the malady was due to electrical discharges in the atmosphere had many supporters.

But it was not until 1878, one hundred years after the first record of the trouble, that the true cause of the disease was discovered. In that year Burrill¹ (9) of the University of Illinois, at a meeting of the Illinois State Horticultural Society stated his belief that the real cause of the disease was due to bacteria. In 1880, after performing a series of experiments by inoculating healthy branches with wilted material from diseased ones, he announced his results at the Boston Meeting of the American Association for the Advancement of

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1. Trans. Ill. Hort. Soc. 12, 79-80. 1878.

Science¹ (11). His experiments evidently proved that the disease was due to bacteria, but, in spite of this fact, the theory was not at first accepted.

In 1881, Arthur² (1), then of the New York Experiment Station, after a series of experiments proved that the disease is infectious and may be communicated to healthy limbs by inoculation. During the years 1884-1886 Arthur carried on an extensive series of experiments on the spread of the disease and methods for its control.

Some work has been done by various scientists on the effect of the blight bacteria on the other varieties of fruit. Jones³ (27) states that the blight of the Plum is due to the same bacillus as the pear blight, and Arthur, Burrill, and others claim that the Twig blight of the apple and ^hWetzel⁴ (41) that the "Blight Canker" of Apple are all due to the same organism.

DISTINGUISHING CHARACTERS OF THE DISEASE IN THE APPLE

So many descriptions of the distinguishing characters and the appearance of the disease of the pear caused by Bacil-

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1. Proc. Amer. Assoc. 19: 583-597. 1880.
2. Amer. Nat. 19: 1177-1185. 1885.
3. Centr. f. Bakt. 9. 835-841. 1902.
4. New York (Cornell) Expt. Sta. Bull. No. 236. 102-138. 1906.

lus amylovorus (Burr.) Trevisan have appeared in literature that an account of them need not be given in this paper, as they are probably very familiar.

The apple disease studied agrees very closely with that which Whetzel¹ (41) has termed the "Blight Canker", and the following is taken from Whetzel's ¹ (41) description of the appearance of the disease: "The cankers have also been observed in the upper crotches and on the sides of the large limbs in old trees. Spreading from these points of infection, the disease may gradually involve the entire limb, causing its death. It is on young trees that the canker is most dangerous for, unless promptly attended to, the disease is almost certain to result in the death of the entire tree. In young trees with smooth bark the cankers are easily detected even in their first stages. They appear as discolored and somewhat sunken areas, the margin along the advancing front being usually slightly raised or blistered. The tissue in actively spreading cankers is of a darker green than the healthy bark and is very watery or sappy. After a short time the diseased tissue begins to turn brown and dry out. Unless in a very active state of progress the margins are very distinct, marked by a crack where, in drying, the diseased tissue has separated from the healthy bark. The surface is smooth never checked or roughened or beset with pustules or pimples, except

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1. N. Y. Expt. (Corn.) Sta. Bull. No. 236. 1906.

in the old cankers, where, after a time, rot fungi gain entrance, and, thriving in the already dead tissues produce their fruit bodies on the surface. . . . The diseased bark is usually killed to the wood, to which it clings tenaciously the first season. It gradually decays, however, and falls out, leaving the wood bare and exposed." The cankers vary in size from a few inches to three or four feet in length and are quite variable in width. Those in the crotches of the tree retain the water for some little time and the wound fungi entering the dead tissues, gradually spread, if conditions are favorable, and eventually cause the death of the entire branch or limb, and perhaps that of the tree.

It was at first thought that the cause of the trouble would be found in the roots of the trees, but a careful examination of them was made, and they were found to be in a healthy condition.

Under the microscope, however, sections which were cut from the darkened areas of bark on the limbs, where the tree appeared to be diseased, showed large numbers of bacteria wedged in between the cells of the starch sheath and around the bast bundles. These colonies or groups of bacteria are very abundant throughout the inner cortical tissue, especially so just above the place where the diseased area blends with the healthy bark.

After a careful removal of the outer bark with a sterilized knife, bits of the diseased inner cortical area at

the points of advancement of the disease including the cambium and bits of the outer wood were used in making poured plates. Pure cultures were easily obtained because of the abundance of bacteria within the tissues of the host, all of which proved to be of one kind.

AMOUNT OF DESTRUCTION

The amount of destruction caused by the bacteria in the apple is enormous. Whetzel¹ states that in New York state "examination of several orchards showed not less than 95 per cent of the trees were diseased. In one orchard of originally some four hundred trees which began to go out in 1903 less than fifty were alive in June, 1905, and but few of these were entirely free from canker." He also found young orchards had suffered almost total destruction while old trees did not seem to have suffered to any considerable extent.

MORPHOLOGIC CHARACTERS OF THE TWO ORGANISMS

1. Bacillus amylovorus (Burr.) Trev.

A bacillus, usually single, often in pairs, in rapidly multiplying cultures, occasionally in chains of three or four cells, but this is unusual; from 0.75μ - 1.25 - 1.5μ in

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1. N. Y. Exp. (Corn.) Sta. Bull. No. 236. 1906.

length by a little over half as wide; neither capsule, flagella, nor spores observed. Stains readily with either watery or alcoholic solutions of the analin stains, also by Gram's Iodine Method.

Movement: Among many motionless organisms some exhibit an oscillatory motion and a small per cent have distinct ciliate movement, sometimes very rapid, indicating a rapid progress in an undulating course so that they pass across the entire field whirling vigorously upon their short axis. Movement ceases after a few moments. Examination made from 36 hour beef bouillon culture with 1/6 in. objective.

2. Organisms obtained from the Grimes' Golden and Willow Twig varieties. As these organisms appear identical morphologically, they are described as a single species.

Short, oblong-oval to almost rod-shaped cells from 1-2 μ in length and a little over half as thick; usually single but occasionally double cells found. Stain readily in any of the common bacterial stains, especially well in Carbol-fuchsin and Methylene blue. Neither capsule, flagella, nor spores demonstrated.

Movement: Similar to that of Bacillus amylovorus, but a larger per cent exhibiting ciliate motion. Both examinations made from 36 hour cultures in beef bouillon.

Five descriptions of the morphologic characters of Bacillus amylovorus (Burr.) Trevisan have thus far appeared

in literature. Two of them, those of Burrill¹ (15) and Arthur² (2) appeared in the early eighties shortly after its discovery as the cause of pear blight and are given in full below; the other three, those of Chester³ (17) in 1900, Jones⁴ (27) in 1902, and Whetzel⁵ (41) in 1906, were based upon the organisms infesting different host plants. Chester's description is from a pure culture of the organism isolated from a blighted pear twig; Jones' description from cultures from blighted pear and plum shoots, and Whetzel's from the cultures obtained from both pear and apple.

Burrill's¹ original description as published in 1883 is as follows: "Cells oval, single or united in pairs, rarely in fours, never in elongated chains, imbedded in an abundant mucilage which is very soluble in water; movements oscillatory; length of a separate cell .00004 in. to .000056 in.; width .000028 in.; length of a pair .00008 in.; of four united about .00012 in."

Arthur's description published in 1886 is as follows: "The form of this species of bacteria is very constant, under all conditions. The single cells are from oval to roundish

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1. Amer. Nat. 17. 319. 1883.
2. Proc. Phila. Acad. Sci. 322-341. Pl. III. 1886.
3. Del. Rept. 12: 38-46. 1900.
4. Centr. f. Bakt. 9: 835-841. 1902.
5. N. Y. (Corn.) Expt. Sta. Bull. No. 236. 1906.

ovoid, and only vary by slight changes in the ratio between their length and breadth. They are 1 to $1\frac{1}{4}\mu$ long, by $\frac{1}{2}$ to $\frac{3}{4}\mu$ broad, and quite colorless. For the most part, they exist as single independent cells, but may often be found in pairs, especially when still multiplying, and in rare instances are united into a series of four or even more, but never extend into chains during rapid vegetation; in rich nutritive media, the movements reach a stage of extreme activity. The appearance is what is termed swarming, in which the bacteria move rapidly back and forth, in and out among each other, but never in a straight line to any distance."

These two early descriptions agree with one another in general; in the former we have the measurements in inches, an unusual method at the present day, while Arthur gives his in micra.

PHYSIOLOGIC CHARACTERS

As stated above, five descriptions of Bacillus amylovorus (Burr.) Trevisan have thus far appeared in literature, and four of the five are based on cultural characters. In all four of these descriptions the authors have stated the cause of the trouble was due solely to Bacillus amylovorus (Burr.) Trevisan and have "established beyond a doubt the identity of the organisms causing the canker on the bodies and limbs of apple and pear trees and fire and twig blight of

these trees¹ ", and that of the plum and pear blight to be the same.

In their cultural observations they have found practically identical growth of all the organisms in the various kinds of media employed. In this phase of the work, however, present investigations lead to different conclusions. Under the variety of media employed separate accounts of the growth of the three organisms under discussion will be given in full in so far as any variation between them appears.

The study of these cultural characters has extended over a period of five months, and, while observations upon Bacillus amylovorus (Burr.) Trevisan differ in some respects from those of previous writers, it must be borne in mind that the personal equation is an important factor in all such bacteriological work, for the differences in the composition of the media employed cause differences in cultural features, and the ability to detect various characteristics varies with different observers.

The differences in the characteristic growth of Bacillus amylovorus (Burr.) Trevisan and that of the organisms infesting the Grimes' Golden and Willow Twig varieties of apple cannot be traced to this cause, however, for enough of each medium was prepared at one time to grow all three organisms in parallel cultures under the same conditions. Observa-

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1. N. Y. (Corn.) Expt. Sta. Bull. 236. 1906.

tions and records were made daily. The cultures were grown in a bacteriological incubator kept at a temperature of 28 to 30 degrees centigrade.

The stock culture of Bacillus amylovorus (Burr.) Trevisan used in carrying on these experiments was an agar slant culture obtained from the Bacteriological Laboratory of the University of Illinois.

Several pure cultures of the organisms infesting the Grimes' Golden and Willow Twig varieties were obtained by the usual agar plate method. Every precaution being taken to avoid contamination, the pure cultures thus obtained from the diseased areas were each grown in the different kinds of media described below, and a careful record made of the growth and characters of each culture. From six to ten cultures of each of the three organisms were made with the various kinds of media.

CULTIVATION IN SYNTHETIC MEDIA

Uchinsky's Solution

B. amylovorus. In two days fluid becomes quite cloudy in the upper portion of the tube and growth evenly distributed throughout about the fifth day. A thin film forms at its surface. The liquid becomes somewhat gelatinous after ten days. Very little sediment.

Organism from Grimes' Golden. Very little perceptible growth even after five or six days. Sediment very slight and liquid only faintly cloudy; no film formed.

Organism from Willow Twig. Growth slow and very weak becoming noticeable in six or eight days. Very faintly cloudy and little sediment formed.

Cohn's Nutrient Solution

B. amylovorus. Very rapid growth giving liquid a cloudy appearance within twenty-four hours. Light film of a grayish white color formed at the surface. Little sediment and of a grayish white color.

Organism from Grimes' Golden. No growth observed and medium apparently unchanged.

Organism from Willow Twig. Same as the preceding.

Prazmowski's Culture Fluid

B. amylovorus. No growth.

Organism from Grimes' Golden. Very weak growth becoming apparent after three or four days.

Organism from Willow Twig. Very weak growth.

Naegeli's Solution

B. amylovorus. No growth.

Organism from Grimes' Golden. No growth.

Organism from Willow Twig. No growth.

NON-SYNTHETIC MEDIA

FLUID CULTURES

Beef Bouillon.

B. amylovorus. Becomes faintly cloudy after twenty-four hours and quite cloudy after forty-eight hours. Very slight pellicle formed with a moderate amount of grayish-white sediment. Tendency to clear after about three weeks. Grayish color.

Organism from Grimes' Golden. Good growth giving medium a cloudy appearance in from twenty-four to thirty-six hours. Film forms at surface about the third day. Small amount of sediment of a brownish-gray to grayish white color, usually beginning to form about the fourth day.

Organism from Willow Twig. Rapid growth causing medium to become cloudy in about twenty-four hours. Film forms at about third day, also sediment begins to fall. General characters very closely resemble those of the organism from Grimes' Golden. Color of a brownish to yellowish gray. Sediment dirty white.

Potato Bouillon

B. amylovorus. Medium becomes cloudy throughout within twenty-four hours. Growth very rapid, more active near surface. Sediment very abundant and of grayish appearance. In old cultures (three weeks), liquid becomes clear above and large amount of sediment below. Very thin film is formed at the surface.

Organism from Grimes' Golden. Becomes quite cloudy within twenty-four hours. Growth rapid, film forming at surface and sediment forming on the third or fourth days. General color grayish, sediment brownish-gray. Shows tendency to clear in old cultures.

Organism from Willow Twig. Cultural characters practically identical with those of the organism from the Grimes' Golden.

Milk

B. amylovorus. Thickens a little at surface in three or four days. No color, little whey formed. Slightly alkaline to litmus.

Organism from Grimes' Golden. Thickens at surface on second day becoming quite thick on fourth or fifth day. Large amount of whey-like liquid formed above the thickened portion which gradually settles to the bottom where later digestion takes place. Color a brownish straw. Strong alkaline reaction to litmus.

Organism from Willow Twig. Very similar in cultural characters to organism from Grimes' Golden. Growth a little slower.

Litmus Milk

B. amylovorus. Very weak growth. Litmus reddens a very little. Faintly alkaline.

Organism from Grimes' Golden. Very rapid growth. Milk thickens rapidly and in from three to four days the milk has lost its lavender color and appears as a brownish whey-like liquid which digests the thicker portion. Very marked alkaline reaction.

Organism from Willow Twig. Very similar to preceding but slower growth.

SOLID MEDIA

Gelatin Plates

B. amylovorus. Growth slow; colonies becoming visible in from two to five days. The colonies are round, somewhat granular and white in color with entire margins. Surface colonies commence to liquefy the gelatin in about ten days and small pits about the colony are formed.

Organism from Grimes' Golden.)	) Very similar to the
Organism from Willow Twig.)	
preceding except	
that liquefaction takes place more rapidly and starts from	
two to three days earlier.	

Gelatin Stab Cultures

B. amylovorus. Growth slow. On surface, thin, irregular, and with white margin, the center being somewhat granular. Liquefaction rather slow and crateriform.

Organism from Grimes' Golden. Just about the same as the preceding. Somewhat granular along the line of puncture. Liquefaction slow and usually near the surface.

Organism from Willow Twig. Growth similar to that of organism from Grimes' Golden except that liquefaction appeared to be more rapid than in the case of either of the other organisms.

Agar Slant

B. amylovorus. Growth a white, glistening, somewhat slimy streak, showing plainly in twenty-four hours. Spreads quite rapidly and has an irregular outline. Rather thick along the edge as in the gelatin cultures.

Organism from Grimes' Golden. General appearance to *B. amylovorus*. Not quite as slimy but somewhat thicker growth which is very rapid and quickly spreading over the surface, giving it a somewhat vitreous lustre.

Organism from Willow Twig. Quite similar to organism from Grimes' Golden in its physiologic characters.

Agar Stab

B. amylovorus. Fine growth at surface and along entire length of puncture. Growth rapid and penetrating medium to about 3-5 mm. from puncture.

Organism from Grimes' Golden.)	Both showed fine sur-
Organism from Willow Twig.)	face growth but not

very vigorous along line of puncture. Does not penetrate media to such an extent as *B. amylovorus*.

Agar Plate Culture

B. amylovorus. Colonies evident in about forty-eight hours. Surface colonies rather minute, round, entire to very slightly wavy and of a granular appearance, somewhat shining and glistening. Deep colonies round, rather opaque, and glistening.

Organism from Grimes' Golden. Surface colonies small, round, white, elevated, and of a slimy appearance. Margins wavy and rarely entire, cloudy with distinct white central portion. Buried colonies round, yellowish, and opaque.

Organism from Willow Twig. Very similar to the physiologic characters of the organism from Grimes' Golden. Both organisms have a rapid growth.

REMARKS

During the study of the diseases of the apple careful search was made to find mycelium in the roots of the trees which showed signs of the disease, and in darkened areas of the bark, but none was found. On the other hand, bacteria were present in large numbers in the inner cortical layers of the bark, thus showing the diseases were of bacterial origin. The destructiveness of the diseases in New York state shows that, if they spread, they are likely to be some of the worst with which the orchardists will have to combat.

When bacteria were found so abundantly within the inner cortical layers of the bark, it was decided to make a study of these organisms, especially regarding their cultural characteristics, and the results are noted above.

Attention is called to the differences in growth of the three organisms in Uschinsky's, Cohn's Nutrient, and Prazmowski's Culture fluids. In spite of the fact that their morphologic characters are so very similar the difference of the growth of the three organisms in the above named fluids would tend to show a difference in species.

In the non-synthetic cultures the most striking differences in growth of the three organisms were found in the Litmus milk cultures. Here again is almost positive evidence that the organisms are not identical species. Before definite conclusions are drawn, however, a long series of inoculation

experiments with the three organisms will be required to settle finally the question as to whether they constitute one or more species.

The change in the nomenclature from Bacillus amylovorus (Burr.) de Toni to Bacillus amylovorus (Burr.) Trevisan is made from the statement found on page 984 of Vol. VIII of Saccardo's Sylloge Fungorum which indicates that Trevisan and not de Toni is the author of the change from Micrococcus to Bacillus.

ACKNOWLEDGEMENTS

The writer wishes at this time to express his thanks to Dr. T. J. Burrill, under whose direction the work was carried forward, for his kind suggestions and assistance in connection with the study of the organisms. To Dr. H. A. Gleason and Mr. E. S. Reynolds thanks are due for many timely suggestions and for their kind criticism of the manuscript.

FORMULAE OF CULTURE MEDIA AND STAINS EMPLOYED IN THIS STUDY

NON-SYNTHETIC MEDIA

Standard Peptonized Bouillon.

Liebig's Meat Extract - - - - - 3 grs.
Distilled Water - - - - - 1000 cc.
Witte's Peptonum Siccum - - - - - 10 grs.
Titrated to normal.

Agar Preparations.

Powdered agar agar - - - - - 3 grs.
Standard Bouillon - - - - - -300 cc.
No white of egg added. Titrated to normal.

Powdered agar agar - - - - - 4 grs.
Standard Bouillon - - - - - -600 cc.
White of egg neutral to litmus.
Titrated to normal.

Agar agar strips - - - - - 5 grs.
Soaked overnight in dilute acetic acid and
washed for two hours in running water in the
morning.

Bouillon - - - - - 500 cc.

White of egg neutral to litmus

Titrated to normal

Gelatin Preparations

Bouillon - - - - - 325 cc.

Gelatin (sheet) - - - - - 30 grs.

Soaked two hours at room temperature, steamed five minutes, cooled, white of egg added, steamed thirty minutes, and filtered.

Bouillon - - - - - 300 cc.

Gelatin - - - - - 35 grs.

Soaked two hours at room temperature. Steamed, white of egg added, and titrated, steamed, and filtered.

Potato Bouillon

Cleaned two potatoes with 1/1000 Corrosive Sublimate. Pared and boiled in 3 times as much distilled water at 70° Centigrade. Filtered and sterilized.

Milk

A flask of milk was obtained from the Dairy Department of the University of Illinois. Milk was free from cream and was sterilized in an autoclave as directed by Smith¹ in his "Bacteria in Relation to Plant Diseases".

Litmus Milk

One part of Merck's lime-free c. p. blue litmus, saturated solution to 50 parts of milk. Litmus milk had lavender color.

SYNTHETIC MEDIA

Ushinsky's Solution

Distilled water - - - - -	500 cc.
Glycerine - - - - -	20 cc.
Ammonium lactate - - - - -	3 cc.
Sodium chloride - - - - -	2.5 gr.
Calcium chloride - - - - -	.05 gr.
Magnesium sulphate - - - - -	.2 gr.
Dipotassium phosphate - - - - -	.12 gr.
Sodium asparaginate - - - - -	1.5 gr.

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1. Smith, E. F. , Bacteria in Relation to Plant Diseases.
Page 46. 1905.

Cohn's Nutrient Solution

Distilled water - - - - -	500 cc.
Acid Potassium phosphate - - - - -	2.5 grs.
Magnesium sulphate - - - - -	2.5 grs.
Neutral ammonium tartrate - - - - -	5.0 grs.
Potassium chloride - - - - -	.25 grs.

Prazmowski's Culture Fluid

Dipotassium phosphate - - - - -	2.5 grs.
Magnesium sulphate - - - - -	2.5 grs.
Ammonium chloride - - - - -	2.5 grs.
Calcium chloride - - - - -	.25 grs.
Distilled water - - - - -	500 cc.

Naegeli's Solution

Calcium chloride - - - - -	0.05 grs.
Magnesium sulphate - - - - -	.1 gr.
Dipotassium phosphate - - - - -	.5 gr.
Ammonium tartrate - - - - -	5.0 grs.
Distilled water - - - - -	500 cc.

Ehrlich's Anilin-water Gentian Violet.

Alcoholic Solution of Gentian Violet (saturated)	5 cc.
Anilin water - - - - -	- 100 cc.
Does not keep well.	

Ziehl's Carbol Fuchsin

Fuchsin (basic) - - - - - 1 gr.
Absolute alcohol - - - - - 1 cc.
Carbolic acid (5% solution in water) 10 cc.

Loeffler's Alkal. Methylene Blue

Sat. solution of Methylene Blue - - - 3 cc.
Distilled water 10,000 parts)
Caustic potash 1 part) - - - 10 cc.
"An excellent stain."

Gram's Solution

Iodine - - - - - 1 gr.
Potassium iodide - - - - - 2 grs.
Distilled water - - - - - 300 cc.

Hauser's Spore Stain

Pass cover glass quickly through the flame three times. Drop on carbol-fuchsin and heat for five minutes over flame, renewing the stain as it boils away. Nearly decolorize in 5 % solution of acetic acid. Wash very thoroughly. Counter-stain with dilute watery solution of Methylene blue or with Loeffler's Methylene Blue.

Ribbert's Capsule Stain

Water - - - - - 10 cc.

Alcohol - - - - - 5 cc.

Glacial Acetic - - - - - 1.25

Warm and add dahlia to saturation.

Covers are barely touched to this stain, and
are then washed in H₂O.

Stain keeps well.

Loewits Flagella Stain

(1) Mordant.

Distilled H₂O - - - - - 1 cc.

Tannin - - - - - 25

Dissolve and filter through two thicknesses
of filter paper, then add

Saturated solution Cu SO₄ - - - - - .5 cc.

Saturated alc. sol. basic fuchsin - - - .1 cc.

(2) Expose cover to Ehrlich's anilin water gen-
tian violet 1-5 minutes. Wash thoroughly in H₂O
and if this is not sufficient plunge for a moment
in 50% alcohol or acid alcohol (1 drop of 3% HCl)
alc. in 3-4 cc. of 60% alc. Mordant and stain
must be made up each time.

LITERATURE

In the accompanying list of references no attempt has been made to record all the various articles on the subject, but rather those which have the most important bearing upon it. They have been arranged alphabetically by authors and numbered, the numbers in the text referring to the numbers preceding the author's name in the list.

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EXPLANATION OF PLATES

The accompanying photographs are of diseased apple trees, of the Willow Twig variety, in the orchard of Senator H. M. Dunlap located at Savoy, Illinois.

Plate I Fig. 1 General view of medium sized tree. Note absence of foliage on the limbs affected by the blight organism.

Fig. 2 General view of tree killed by the disease. The large limb on the ground, badly diseased, was broken by the wind.

Plate II Fig. 1 View showing the central portion of tree affected by the disease, the lower limbs thus far unaffected.

Fig. 2 A diseased branch.

Plate III Fig. 1 "Crotch canker". Note the absence of bark on the limb to the right which has been killed by the canker. The dry bark has separated from the wood and broken off. All the other limbs are diseased.

Fig. 2 Large crotch canker. The limbs to the right show the separation of the dry bark from the wood, those on the left show the characteristic darkened areas of active cankers.

Plate IV Fig. 1 A canker on a horizontal limb.

Fig. 2 Characteristic effect of cankers on limbs. Note the darkened areas on the upper limb

which show the appearance of an active canker.

Plate V Fig. 1 Rather advanced stage of the disease. Left hand side of the branch is badly affected, the extreme right hand side is still healthy. Note the cracking and separation of the bark between the healthy and diseased areas.

Fig. 2 Another view showing the cracks in the bark between healthy and diseased areas.

Plate I.



Fig. 1.



Fig. 2.

Plate II.



Fig. 1.



Fig. 2.

Plate III.



Fig. 1.



Fig. 2.

Plate IV.



Fig. 1.



Fig. 2.

Plate V.



Fig. 1.



Fig. 2.





